

XV. CONSTITUENTS OF THE BARK OF *ZANTHOXYLUM MACROPHYLLUM*, OLIVER.

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THE chemistry of *Zanthoxylum* species is in a confused state partly owing to the doubtful authenticity of the material examined by various investigators, but chiefly due to the confused botanical synonymy of the genus. This difficulty has arisen largely through the transfer by Engler [1895] of a large number of species from *Zanthoxylum* to the genus *Fagara* so that closely allied species have been examined under apparently distinct names, with the result that a number of substances have been described as new, which are so similar to substances already known as to warrant the assumption that they are identical.

In the course of the present investigation the author has had occasion to make as complete an examination as possible of the literature relating to the chemistry of *Zanthoxylum* and *Fagara* species and as the results present some interesting points they are briefly summarised here. The following list of nine species includes all that have been examined and from which characteristic and well-defined substances have been isolated:

(1) *Zanthoxylum brachyacanthum*, F. Muell (*F. brachyacanthum*, Engler) contains the alkaloids 1- α -canadine methochloride, $C_{21}H_{24}O_4NCl$, and γ -homochelidonine [Jowett and Pyman, 1913].

(2) *Z. Ochroxylon*, D. C. (*F. monophylla*, Lam.) contains two alkaloids; $C_{24}H_{23}O_6N$, and a second for which no formula is given [Leprince, 1911].

(3) *Z. Clava-Herculis*, Linn. (*Z. carolinianum*, Lam.; *F. caroliniana*, Engler). According to Perrins [1862] this contains berberine, and more recently Gordin [1906] has isolated from it xanthoxylin S, $C_{14}H_{12}O_4$ or $C_{21}H_{18}O_6$. There seems to be some doubt as to the authenticity of the bark used by Perrins, and Wehmer suggests that it was really derived from *Z. caribaeum*, Lam., which Perrins himself suggests as a synonym for the species he used though according to the Index Kewensis the two are distinct species.

(4) *Z. senegalense*, D. C. (*F. xanthoxyloides*, Lam.) contains an alkaloid $C_{21}H_{23}O_4N$ or $C_{20}H_{17}O_4N$ and a neutral substance $C_{10}H_{10}O_3$ or $C_{14}H_{14}O_4$ [Giacosa and Soave, 1889], caprinamide, xanthotoxin $C_{12}H_8O_4$, fagarol $C_{20}H_{18}O_6$ [Priess, 1911] and fagaramide $C_{14}H_{17}O_3N$ [Thoms and Thümen, 1911].

(5) *Z. macrophyllum*, Oliver (*F. macrophylla*, Engler) contains fagaramide and lupeol (see below).

(6) *Z. Budrunga*, D. C. (*F. Budrunga*, Roxb.) contains xanthosterin $C_{23}H_{39}OH$ [Dieterle, 1919].

(7) *Z. flavum*, Vahl. (*F. flava*, Kr. et Urb.) contains a lactonic substance $C_{11}H_{10}O_3$ and an ether-lactone $C_{14}H_{12}O_3$ [Auld and Pickles, 1912].

(8) *Z. americanum*, Mill (*Z. fraxineum*, Willd.) contains xanthoxylin N, $C_{15}H_{14}O_4$ [Gordin, 1906].

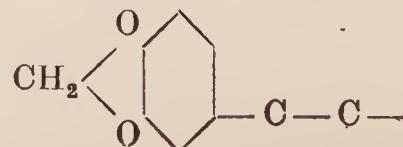
(9) *Z. piperitum*, D. C. contains a crystalline substance $C_{10}H_{12}O_4$ [Stenhouse, 1854, 1857].

The presence of alkaloids has been recorded in all the above mentioned species, but the amount present appears generally to be small except in the four cases (*Z. brachyacanthum*, *Z. Ochroxylon*, *Z. senegalense* and *Z. Clava-Herculis*) in which they have been isolated and characterised. The other definite constituents fall into three classes: lactones, phytosterols and amides.

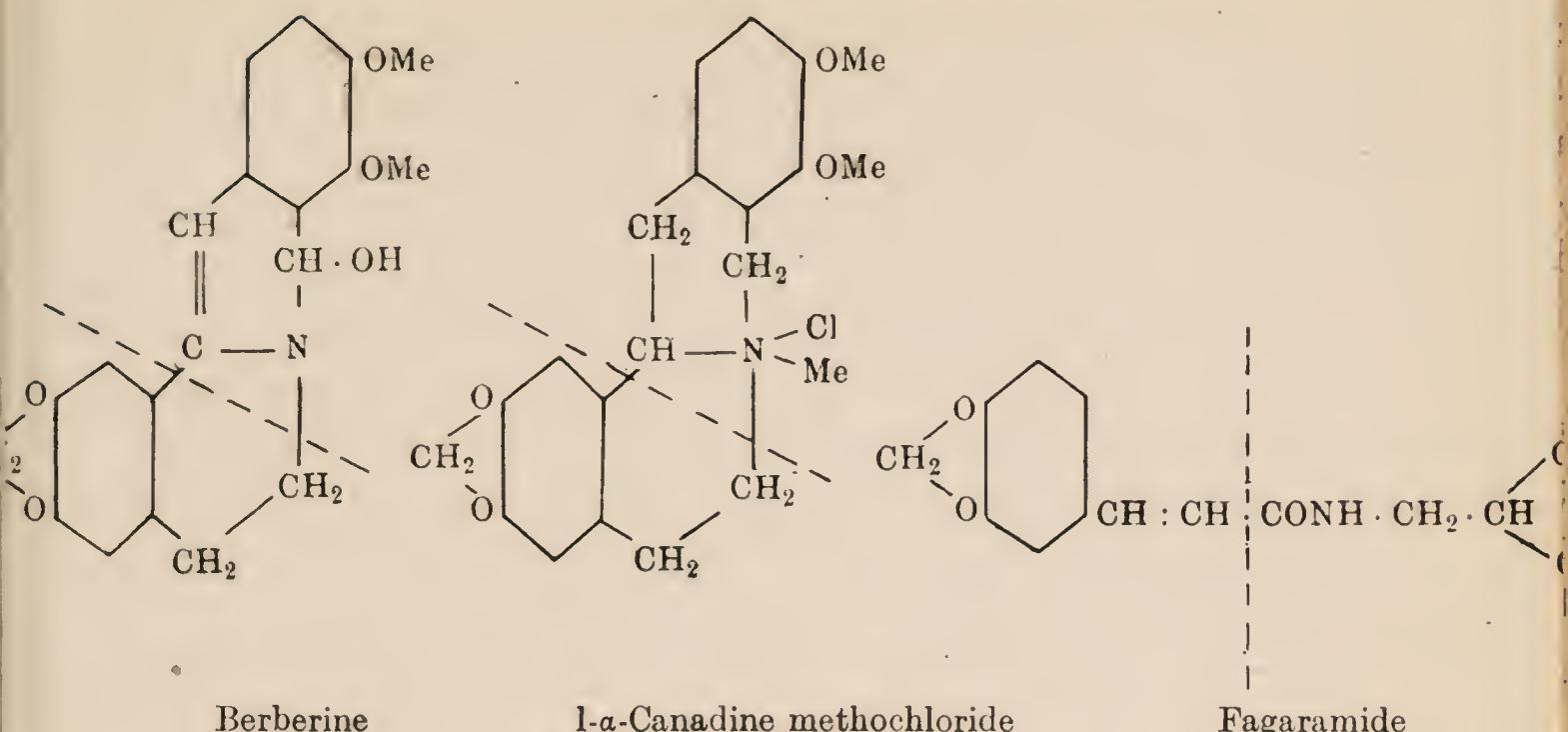
Thoms and Thümen [1911] have suggested that the neutral substance (M.P. 120°) isolated by Giacosa and Soave from *Z. senegalense*, to which they assigned the formula $C_{10}H_{10}O_3$ or $C_{14}H_{14}O_4$ was in all probability fagaramide $C_{14}H_{17}O_3N$. The name xanthoxylin has been applied to three apparently distinct substances two of which have been distinguished by Gordin [1906] as xanthoxylin S and xanthoxylin N. It seems possible that xanthoxylin S from *Z. Clava-Herculis*, for which Gordin proposed the formula $C_{14}H_{12}O_4$ or $C_{21}H_{18}O_6$, is fagaramide, since, although it is not recorded as containing nitrogen, this constituent is not mentioned as having been sought for, and the composition and constants of the substance are otherwise in close accord with those of fagaramide. Xanthoxylin N, $C_{15}H_{14}O_4$ [Gordin, 1906] from *Z. americanum*, Mill and the lactonic substance $C_{11}H_{10}O_3$ [Auld and Pickles, 1912] from *Z. flavum*, Vahl. are similar in properties, and may be identical.

Again the melting points of xanthosterin $C_{23}H_{39}OH$ [Dieterle, 1919] from *Z. Budrunga*, D. C. and its derivatives are in striking agreement with those of lupeol now shown to be present in *Z. macrophyllum*. The analytical figures are not in good agreement, but this is not surprising in view of the difficulty of purifying phytosterols; thus the formula for lupeol is variously given in the literature as $C_{26}H_{42}O$, $C_{30}H_{50}O$, and $C_{31}H_{50}O$, the second being probably correct.

It is interesting to note that fagaramide occurs in the two species *Z. senegalense* and *macrophyllum*, which are both West African, and as noted below are closely allied. A further point of interest lies in the fact that berberine, 1- α -canadine methochloride, and fagaramide which have been isolated from four of the species mentioned above contain the grouping



in common.



Berberine

1- α -Canadine methochloride

Fagaramide

Of the nine species examined all but two, viz. *Z. americanum* and *Z. piperitum*, were included in Engler's transfer. No marked difference can be traced between the components—so far as they have been ascertained—of these two species and those found in Engler's Fagara species, so that the chemical evidence appears at present to be of no value in deciding for or against the validity of Engler's view.

Zanthoxylum macrophyllum.

Zanthoxylum macrophyllum, Oliver (*Fagara macrophylla*, Engler) of the natural order Rutaceae is a tree 30 or 40 feet high, with either spreading or ascending prickles on its leaf-stalks, and leaves composed of numerous leaflets 6 to 8 inches long. It is closely related to but quite distinct from *Fagara xanthoxyloides*, Lam. (*Zanthoxylum senegalense*, D. C.); the latter does not exceed 20 feet in height, has recurved prickles and leaves with not more than nine leaflets, 2 to 3½ inches long. Both species occur in Upper Guinea. The present investigation shows that the bark of *Zanthoxylum macrophyllum* contains fagaramide, previously isolated by Thoms and Thümen [1911] from *Fagara xanthoxyloides* and thus further emphasises the close relationship of the two species, already indicated by their structural similarity.

The material used in the present investigation came from the Gold Coast Colony and was kindly supplied by the authorities of the Imperial Institute. The bark is said to be used by the natives as an analgesic, especially in cases of toothache and rheumatism. It produces a tingling sensation in the mouth when chewed.

EXPERIMENTAL.

The material contained 11.2 % of moisture and 4.7 % of ash. Only traces of alkaloid could be detected by the usual reagents. The finely ground bark gave the following percentages of extract on exhaustion in a Soxhlet apparatus with solvents in the order named: petroleum (B.P. 35–60°), 1.46; ether, 0.95;

chloroform, 1·48; alcohol, 12·07; water, 4·80. Of these extracts the first and second appeared to contain all the substance producing the tingling sensation, to which presumably the alleged physiological action is due.

Petroleum extract. The ground bark (32 kilos) was extracted with hot petroleum (B.P. 35°–60°). On standing this deposited a sparingly soluble substance amounting to 60·4 g. or 0·18 % by weight of the bark, which proved to be fagaramide (see below). The mother liquors on concentration deposited in several crops 85·9 g. of a phytosterol, which was shown to be lupeol. The residue of the petroleum extract was of a resinous character and contained the substance producing the tingling sensation when applied to the tongue. Attempts to isolate this substance in a pure state were unsuccessful.

Ether extract. The ground bark after extraction with light petroleum was exhausted with hot ether, the solution shaken with aqueous solution of potassium hydroxide, separated and the solvent distilled off. The residue on extraction with petroleum yielded a further quantity of fagaramide, whilst the resinous active substance remained in solution.

Identification of Fagaramide.

The crude fagarainide, obtained as described above, was repeatedly recrystallised from ethyl acetate, and so obtained in colourless six-sided plates, which softened at 105° and melted at 119·5° (corr.). It gave Gaebel's test [1910] for the methylene di-oxy group, and readily absorbed two atoms of bromine.

	Found	Calculated for fagaramide, C ₁₄ H ₁₇ O ₃ N
C	67·8, 67·6	68·0
H	7·0, 6·8	6·9
Mol. weight	231, 241	247·2

Fagaramide is hydrolysed with difficulty by alkalis or acids. It was found most convenient to obtain the acid hydrolytic product, piperonylacrylic acid, by alkaline hydrolysis, and the basic hydrolytic product, *iso*-butylamine, by acid hydrolysis.

Alkali hydrolysis. 0·5 g. of fagaramide was boiled for four hours with a mixture of 12 g. of 50 % aqueous sodium hydroxide and 12 cc. of methyl alcohol. The mixture on dilution with water yielded 0·33 g. of unchanged fagaramide. The filtrate after acidification yielded to ether 0·13 g. of piperonylacrylic acid. The piperonylacrylic acid (from several hydrolysés) was recrystallised from ether, in which, when dry, it is only sparingly soluble, and obtained in almost colourless needles, melting at 243° (corr.). The melting point was not depressed on mixing with piperonylacrylic acid prepared from piperonal. Found C = 62·1, H = 4·2; calculated for piperonylacrylic acid, C₁₀H₈O₄, C = 62·5, H = 4·2.

Acid hydrolysis. One g. of fagaramide was heated to 130–140° for 14 hours in a sealed tube with 5 cc. of alcohol, 2 cc. of concentrated hydrochloric acid and 3 cc. of water. The tube was washed out with water and ether, the aqueous

solution separated and evaporated to dryness under reduced pressure, where 0.25 g. of crude *iso*-butylamine hydrochloride was obtained. The latter after purification with animal charcoal and recrystallisation from alcohol melted at 178–179° (corr.). The platinichloride changed colour slightly at 204° (corr.), considerably at 225° (corr.) and melted with frothing at 242–243° (corr.). Found Pt = 36.4; calculated for *iso*-butylamine platinichloride, $(C_4H_{11}NH)_2PtCl_6$ Pt = 36.0.

Contrary to the statement of Thoms and Thümen [1911] fagaramide forms salts, which however are easily dissociated. When a solution of fagaramide in chloroform containing dry hydrogen chloride was evaporated at atmospheric temperature under reduced pressure, a crystalline *hydrochloride* melting at 137° (corr.) was obtained. Found HCl = 6.6; calculated for $(C_{14}H_{17}O_3N)_2HCl$. HCl = 6.9.

Identification of Lupeol.

The crude lupeol obtained as already described was repeatedly recrystallised from ethyl acetate and obtained in colourless needles melting at 216° (corr.), and having a specific rotatory power in chloroform of $[\alpha]_D + 31.0^\circ$ ($c = 2.5165$, $l = 1$ dcm., $\alpha_D + 0.78^\circ$). It gave the usual colour reaction with acetic anhydride and sulphuric acid. Found C = 84.6, 84.6, H = 11.6, 11.9; calculated for $C_{30}H_{50}O$, C = 84.4, H = 11.9.

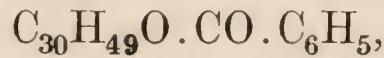
The following derivatives were prepared:

Acetyllupeol, m.p. 220° (corr.), $[\alpha]_D + 44.3^\circ$ in chloroform ($c = 2.5235$, $l = 1$ dcm., $\alpha_D + 1.12^\circ$), found C = 81.8, H = 11.1; calculated for



C = 82.0, H = 11.2.

Benzoyllupeol, m.p. 272° (corr.), $[\alpha]_D + 62.0^\circ$ in chloroform ($c = 2.5020$, $l = 1$ dcm., $\alpha_D + 1.55^\circ$), found C = 83.7, H = 10.0; calculated for



C = 83.7, H = 10.3.

Monobromolupeol, m.p. 183° (corr.); found Br = 15.5, calculated for $C_{30}H_{49}OBr$, Br = 15.8.

Monobromo-acetyllupeol, m.p. 232° (corr.).

It was at first thought that the phytosterol obtained from *Z. macrophyllum* was identical with androsterol obtained by Moore [1909] from *Apocynum androsaemifolium*. No direct comparison could be made as the original reference specimens of androsterol and its derivatives were destroyed in the fire which occurred a few years ago at the Brussels Exhibition. The properties of androsterol and lupeol are however so similar that there can be little doubt of their identity. The phytosterol obtained in this investigation, together with the acetyl and benzoyl derivatives, have been directly compared by means of their melting-points with specimens of lupeol, acetyllupeol, and benzoyllupeol kindly supplied by Prof. van Romburgh, and the identity of the two established.

Examination of the active resin.

The resinous residue obtained as already described exhibited in a marked degree the property of causing a tingling sensation on the lips and tongue, shown by the crude bark when chewed. As this resin could not be induced to crystallise and nothing crystalline could be separated from it by steam distillation or other means it was boiled with $N/2$ potassium hydroxide in alcohol, the alcohol distilled off, and the "unsaponifiable matter" extracted with ether. It constituted 24.4 % of the crude resinous substance and still possessed, apparently undiminished, the property of causing intense tingling of the tongue and lips. It was readily soluble in alcohol or ether and insoluble in water or alkalis. As it could not be crystallised an attempt was made to distil it *in vacuo*. It yielded two fractions, boiling at 240–280°/40 mm., and 280–350°/40 mm. respectively. These distillates though both still physiologically active were less so than the crude unsaponifiable matter. Some decomposition evidently occurs towards the end of the distillation as on opening the flask there was a slight smell of ammonia. The lower boiling of the two distillates when dissolved in alcohol and allowed to stand for some days deposited a small amount of a crystalline substance, which after recrystallisation from ethyl acetate melted at 82° and contained nitrogen. This substance did not produce the tingling sensation.

Mr G. S. Boulger, F.L.S., of the Imperial Institute Staff, to whom the author is much indebted for assistance with the botany of the subject, points out that Bentham and Hooker placed the Linnean genus *Fagara* and several others under the genus *Zanthoxylum*, which thus became a group of some 150 species, and that Engler's action in restricting *Zanthoxylum* to some nine species, and restoring the one Linnean genus *Fagara*, thus appears to be a very slight and conservative revision.

In conclusion, the author desires to express his warmest thanks to Dr T. A. Henry for his advice and criticism throughout the course of the work.

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